Monovalent rotavirus vaccine reduces diarrhoea-associated post-neonatal infant mortality in rural communities in Malawi: a population based birth cohort study

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Abstract

Background

Rotavirus is a major contributor to child mortality. Rotavirus vaccine impact on diarrhoea mortality has been estimated in middle- but not low-income settings, where mortality is high and vaccine effectiveness against hospitalisation is lower. Empirical population-based mortality studies have not been conducted in any setting. Malawi introduced monovalent rotavirus vaccine (RV1) in October 2012.

Methods

We evaluated RV1 impact and effectiveness (VE) against diarrhoea-associated infant (10-51 weeks) mortality using a population-based cohort study of infants born 1st January 2012 to 1st June 2015 in Mchinji, Central Malawi. Individual vaccination status was extracted from caregiver-held records or report at home visits at four months and one year of age. Survival to one year was confirmed at home visit, or cause of death ascertained by verbal autopsy. Impact (one minus mortality rate ratio following-vs-before vaccine introduction) was evaluated using Poisson regression. Among vaccine-eligible infants (born from 17th September 2012), VE (one minus hazard ratio) was evaluated using Cox regression.

Results

We recruited 48,672 live births in Mchinji, among whom 38,518 were vaccine-eligible and 37,570 survived to age ten weeks. VE analysis included 29,085 infants, of whom 108 had diarrhoea-associated death before one year of age. Diarrhoea-associated mortality declined 31% (95% CI: 1, 52; P=0.04) following RV1 introduction. VE against diarrhoea-mortality was 34% (95% CI: -28, 66, P=0.22).

Conclusions

RV1 substantially reduced diarrhoea deaths among infants in this rural, sub-Saharan African setting. These data add considerable weight to the evidence demonstrating the impact of rotavirus vaccine programmes.

Research in context

Evidence before this study

Rotavirus vaccine has been introduced in many high mortality, low income Gavi-supported countries, but mortality impact or effectiveness estimates are lacking from these settings. We searched PubMed using the term ((rotavirus vaccine[Title/Abstract] AND (mortality[Title/Abstract] OR death[Title/Abstract])) NOT "review"[Publication Type] NOT cost-effectiveness[Title]). Title/abstract review of 185 arising citations performed independently by the two first authors excluded review articles and secondary publication of data. Thirteen studies, all from middle-income countries, were identified. Botswana and Panama reported hospitalised case fatality reductions of 48% and 45%, respectively, but did not report on population mortality. All other studies (Bolivia 1, Brazil 5, Mexico 3, combined South American countries 2) used time series analyses of national administrative datasets to estimate mortality reductions following rotavirus vaccine introduction. These studies report infant diarrhoeal-mortality reductions of between 21% and 41%, with higher estimates noted within rotavirus season. No mortality impact data were identified from low-income countries. Prospective, population based studies evaluating rotavirus vaccine impact on mortality have not been published from any country. In southern Malawi, RV1 introduction was associated with 43% reduction in laboratory proven rotavirus infant hospitalisation, with vaccine effectiveness of 64% and was highly cost-effective.

What this study adds

This large population based birth cohort study is the first to report rotavirus vaccine associated infant mortality reductions from a low-income country using the WHO recommended EPI schedule of 6 and 10 weeks, and demonstrates a relationship between coverage and mortality impact gained. In addition, this study demonstrates a possible added

benefit on diarrhoeal mortality of vaccine introduction in the context of enhanced water, hygiene and sanitation improvements.

Implications of all the available evidence

In addition to morbidity impact and high cost-effectiveness, countries with national or localised areas of high diarrhoeal mortality should consider introducing rotavirus vaccines for their survival benefits. Vaccine implementation combined with improvement in water and sanitation may provide maximum impact.

Introduction

Diarrhoea causes 17% of post-neonatal infant deaths globally.[1] Despite impressive survival gains from improved sanitation and case management, in 2013 rotavirus, the greatest contributor to this mortality still caused 215,000 child deaths, 121,000 of these in Africa.[2] Subsequently, with support from Gavi the Vaccine Alliance, many African countries with the highest mortality burdens have introduced live attenuated rotavirus vaccines.[3]

Vaccine impact (population reductions in disease burden following vaccine introduction) and vaccine effectiveness (individual protection afforded by vaccination, henceforth VE) on hospitalized rotavirus gastroenteritis has been shown in high, middle and low-income countries.[4-7] Vaccine efficacy against laboratory proven rotavirus in clinical trials is lower in low-income, high-mortality countries than in high income, low-mortality countries. Therefore to support widespread implementation, evidence of rotavirus vaccine impact on population-level mortality and real-world effectiveness on individual risk of death is crucially important. Vaccine impact on mortality has been demonstrated through analysis of administrative datasets from middle-income countries in Central and South America.[8-10] However, no direct mortality benefit of rotavirus vaccination has been documented at population level from a low-income, high-burden setting.

Malawi, a low-income country in Sub-Saharan Africa, with year-round rotavirus transmission, has made sustained efforts to reduce child mortality and in 2015 had reached the Millennium Development Goal target of reducing child mortality by two thirds from 1990 levels. In Malawi health centres and community based Health Surveillance Assistants (the community healthcare workers/vaccinators in Malawi, henceforth HSA) routinely provide oral rehydration solution and zinc for diarrhoeal disease, and these are widely available. 13-valent Pneumococcal

Conjugate Vaccine was introduced into Malawi's National Immunisation Programme with three doses given at 6, 10, and 14 weeks of age on 12th November 2011. Monovalent rotavirus vaccine (Rotarix™, RV1) at the WHO recommended schedule of 6 and 10 weeks, was introduced on 29th October 2012, without catch-up. We have demonstrated RV1 efficacy (49%, 95% CI: 19, 68), effectiveness (64%, 95% CI: 24, 83) and impact (43%, 95% CI: 18, 61) on severe laboratory confirmed rotavirus gastroenteritis in Malawian infants, and have shown that RV1 is highly cost-effective in this setting.[6, 7, 11, 12]

We aimed to evaluate population-level impact and individual-level effectiveness of RV1 against diarrhoea-associated mortality using a large prospective population-based birth cohort in a rural population in Mchinji district, Central Malawi (Site 1). In order to validate our estimate of RV1 programme impact, we also undertook concurrent impact evaluation in a smaller separate population in Chilumba, Northern Malawi (Site 2, Appendix 1 Fig. 1) .[13] We present the studies at each site in turn.

Methods

Prior to study commencement, extensive community engagement and consultation activities were undertaken with Traditional Authorities, village chiefs, health committees, women's groups, District and Environmental Health Officers, health centre managers and HSAs to ensure the study was welcome in communities and households.

Site 1: Mchinji district - prospective population-based birth cohort

Setting

Site 1 population was 456,516 persons in the 2008 national census, with a crude birth rate of 32 per 1000 population and post-neonatal infant mortality rate of 28 per 1000 live births in 2015.[14, 15] The district is rural and borders Zambia and Mozambique. Its sparsely populated villages and agricultural estates are interspersed with semi-urban trading-centres. The economy is based on subsistence maize farming. Electricity is available in 3.3% of households. [15] This district was the location of a previous cluster randomised trial, with strong community support for research. It had the requisite infrastructure to expand to district-wide mortality surveillance, and allowed us to undertake a large-scale population-based birth cohort study.

Data collection and validation

We conducted a baseline district-wide census in March 2012 to obtain household membership and create community-held household registers. We established prospective household surveillance in 1,832 census-enumerated villages within all 354 HSA clusters by a cadre of 1059 village-based key informants (KIs), who were selected by village health committees. KIs conducted continuous household surveillance and maintained updated paper-based household registers for about 100 households each, recording all pregnancies, birth outcomes and deaths

of children under-5 and of women of childbearing age. KIs were supervised by and reported data monthly to 50 enumerators, who electronically scanned the updated registers. Enumerators conducted home visits of all liveborn infants at four and 12 months of age to record vaccination status and confirm survival. The system was supervised by eight monitoring and evaluation officers (MEOs). Deaths reported by informants were verified and cause of death determined by verbal autopsy (VA) conducted as culturally appropriate at least two weeks after death, by specially trained MEOs using the WHO 2012 VA instrument captured electronically at the household using Open Data Kit software (https://opendatakit.org/).[16] We have published a detailed description of this surveillance system.[13]

Vaccine status was obtained from a scanned image of government issued caregiver-held vaccine record (health passport) and caregiver report (at household visits by enumerators at four and 12 months of age or by MEOs following death). Caregivers were asked directly about receipt and date of each dose of every vaccine to which the child was age-eligible under the National Immunisation Programme. Vaccine status was cross checked against vaccination centre registers in a sub-set of records for quality assurance. Final vaccine status was determined per criteria outlined in Web appendix 4. Additionally, throughout recruitment, interviews with mothers following infant vaccination at randomly allocated clinics compared reported vs recorded vaccine receipt. Throughout recruitment, enumerators collected socio-demographic data on maternal vital and marital status, educational level obtained, and on house, water source and sanitation quality. Quality controls were embedded in the database which automatically triggered field checks in case of error or anomalous runs of data (e.g. no births in a catchment for three months). MEOs met monthly to review data quality and timeliness and address field challenges.

Cohort definitions

Infants surviving at least ten weeks of age who were born between 1st January 2012 and 16th September 2012 constituted the pre-vaccination cohort. Those born between 17th September 2012 (i.e. eligible for 1st dose RV1 on the date of vaccine introduction) and 1st June 2015 constituted the vaccine-age eligible cohort. Impact analysis compared both cohorts, while analysis of individual survival for VE was conducted in the vaccine-eligible cohort only. Live births were followed to one year of age or death or were excluded if they migrated. One year follow-up concluded 1st June 2016. Diarrhoea-associated death was defined as any deceased child whose caregiver reported non-bloody diarrhoea in the illness preceding death upon direct closed questioning at VA.

Analysis

Vaccine programme impact was derived as one minus diarrhoea-associated-mortality rate ratio in the vaccine-eligible cohort vs pre-vaccine-introduction cohort following and prior to vaccine introduction using Poisson regression adjusted for socio-demographic covariates (Table 1). The relative brevity of the pre-vaccine-introduction period at Site 1 precluded adjustment by year. We also performed analysis restricted to January-June, months with known high rotavirus prevalence in Blantyre, Malawi.[17] To examine the relationship between population vaccine coverage and mortality, we Poisson regressed the mortality rate against two-dose vaccine coverage (proportion of 2-dose-eligible infants in the population who actually received both doses) over time and by HSA cluster.[18] For HSA cluster analysis of mortality vs vaccine coverage we also adjusted for cluster-specific means of household-level socio-demographic covariates, but had no data on communal assets such as state of roads or public infrastructure. Plotting of mortality rates over time used locally weighted moving average smoothing (Fig. 2).

Two (vs zero) dose VE was calculated as (one minus hazard ratio) using Cox proportional hazards modelling of diarrhoea-associated death occurring at 10-51 completed weeks of life. Because children may die from causes other than diarrhoea, we also performed competing risk survival analysis. Multivariable modelling was used to adjust for socio-demographic covariates using complete-case analysis (Table 1). We have previously published the primary analysis plan and justification.[19] In case of violation of the proportional hazards assumption and to better understand how VE may be related to age, we conducted fully parametric survival analysis using Royston-Parmar modelling (Fig. 3, panels b, c & d).[20] We examined whether cluster-level determinants influence individual level mortality hazard using random effects hierarchical models.

Sample size

In our sentinel hospital in Blantyre, rotavirus prevalence in severe gastroenteritis is 35% overall and 51% in peak periods; we therefore presumed rotavirus prevalence of 45% in diarrhoea-associated deaths.[6, 21] Given our published VE against hospitalised rotavirus gastroenteritis in Malawian infants is 64%, we assumed VE against very severe rotavirus gastroenteritis (leading to death) would be higher at 70 to 80%. Applying a presumed 76% reduction to the 45% of deaths presumed attributable to rotavirus, gave a VE of 34% against all-cause diarrhoea-associated death. Based on our established surveillance prior to RV1 introduction, we expected 1500 births per month and post-neonatal infant mortality rate (PNIMR) of 18 per 1000 live births, of which six were diarrhoea-associated. We assumed 60% mean vaccine coverage over the recruitment period. Inflating for 12% loss to follow-up, we required 36,293 10-week survivors to obtain 80% power to detect VE≥34%.

<u>Site 2: Validation of Impact Estimate</u>

A Demographic Surveillance Site (DSS) covering 35,000 individuals has operated in the remote lakeside region of Chilumba, Northern Malawi since 2002.[22] Crude birth rate was 30.8 in 2015, post-neonatal infant mortality 15 per 1,000 live births and electricity available in 8.7% of households.[15] This longstanding DSS provided robust data on historical mortality rates in infants prior to vaccine introduction from 2004, and was therefore considered useful for independent impact evaluation. Individual survival analysis was precluded by the small total population. In this site, births, deaths and migrations were reported monthly by village informants and validated in a rolling annual re-census as previously described.[22] Verbal autopsies were conducted at home visit as locally culturally appropriate at least two weeks after death. Socio-demographic covariates and vaccine status were collected on age-eligible children at the time of census visit with vaccination date transcribed from caregiver-held record or caregiver report. Monthly population-based diarrhoea-associated mortality rate among ten-51 week old infants was Poisson regressed against vaccine coverage, adjusting for year to account for long-term trend (Fig. 2 panel b). [23] Unbeknownst to us at planning phase, the Red Cross implemented rapid, widespread and sustained water and sanitation interventions (WASH) across the Site 2 DSS area alongside national vaccine introduction.[24] Site 2 could therefore no longer serve its intended validation function, but afforded an unplanned opportunity to evaluate the combined impact of vaccination with WASH as a post-hoc analysis.

Ethics

Malawi's National Health Sciences Research Committee (#837) and the London School of Hygiene and Tropical Medicine (#6047) provided ethical approval.

Results

Site 1

Cohort Description

We registered 48,672 live births. Of these, the pre-vaccination cohort (born between 1st January 2012 and 16th September 2012) comprised 10,154 infants, among whom 7,818 survived 10 weeks and were included in analysis (Appendix 1 Fig. 2). The vaccine-eligible cohort (born between 17th September 2012 to 1st June 2015) numbered 38,518. Among these 37,570 survived to ten weeks, and 29,085 were included in analysis, of whom 108 died with diarrhoea before one year of age (Fig. 1). Among the vaccine-eligible cohort mean age at diarrhoea-associated death was 34 weeks, and 27 weeks for non-diarrhoea associated death (t-test P<0·001). Two-dose RV1 coverage was 90·6% overall; 90·8% in survivors and 84·3% in deceased infants. Health passports were seen in 90% of infants overall, but ascertainment differed by survivorship; 91% in survivors and 40% among the deceased. Socio-demographic factors were similar among survivors and deceased infants, except for maternal marital status or maternal death (Table 1). Compared with baseline assumptions (see *Sample Size*), in the pre-RV1 period, monthly births were 1,112, PNIMR 18·8, diarrhoea-associated mortality 5·6, and loss to follow-up 18%. Post-hoc exploratory analysis found that infants lost to follow-up had younger (mean age: 25 vs. 27 years) but more educated mothers (15% vs. 12% secondary education) who were more likely to be unmarried (86% versus 89% married) and have slightly better housing quality (11% vs 9% best quality).

Mortality Impact

Prior to vaccine introduction, 44 of 7,818 surviving ten-week olds died with diarrhoea before one year of age (mortality rate [MR] 5·6 per 1000 live births) (Fig. 2 panel a). Among the vaccine age-eligible cohort, 108 of 29,085 surviving ten-week olds died of diarrhoea before one year of age (MR 3·7). Unadjusted and socio-demographically adjusted Poisson regression

estimated vaccine impact on diarrhoea-associated mortality was 34% (95% CI: 6, 53; P=0.03; N=36,900) and 31% (95% CI: 1, 52; P=0.04N=36,770), respectively. For equivalent January-June periods assumed to represent peak rotavirus prevalence, in the post-introduction years 2013 to 2015 the diarrhoea-associated mortality per 1000 was 3·7, 2·1 and 2·6 and respective impact was 44% (95% CI: -3, 70; P=0.06), 67% (95% CI: 31, 85; P=0.003) and 61% (95% CI: 19, 81; P=0.01) (Table 2). All-cause mortality rate reduction post RV1 introduction was 25% (95% CI: 8, 39; P=0.008).

Mortality vs. vaccine coverage

Among 354 HSA clusters of approximately 1,300 persons each,[18] mean post-neonatal infant mortality per 1000 was 12·3 (range 0, 76·9) and diarrhoea-associated mortality was 3·6 (range 0, 64·5). Two-dose vaccine coverage ranged from 63·6 to 100% across clusters; each percentage point greater vaccine coverage was associated with a 1·6% (95% CI: 0·8%, 2·5%) lower diarrhoea-associated mortality rate (Web extra Figure 3). Adjusting for socio-demographic covariates the reduction was 1.1% (95% CI: 0.9%, 1.3%)

Vaccine effectiveness

Among 26,352 fully RV1 vaccinated infants 91 (0·4%) died, while among 1,789 unvaccinated infants ten (0·6%) died (Fig. 3, panel a). Unadjusted and adjusted Cox modelling respectively gave 2-dose VE against diarrhoea-associated mortality of 39% (95% CI: -16, 68) and 34% (95% CI: -28, 66) (Table 1). Adjusting for HSA catchment area using a random effects hierarchical model gave a VE of 36% (95% CI: -24, 67; likelihood ratio [LR] test p<0·001). Analysis of Schöenfeld residuals showed no evidence of violation of the proportional hazards assumption (p = 0·23). Competing risks regression gave a VE of 28% (95% CI: -43, 67). Royston-Parmar model derived VE estimates showed high VE in early infancy which declined

after 6 months of age (Fig. 3 panel c). Further sensitivity analyses and effectiveness against all-cause mortality are presented in Appendix 2.

Site 2

Between 1 January 2004 and 1 June 2015, 15,394 live births were recorded. Of these 3,531 were eligible for RV1 among whom 3,433 survived to 10 weeks. Follow-up was completed on 1 June 2016 for 3,249 infants, of whom 3,235 survived to 1 year. Of the 14 deceased infants, three died with diarrhoea.

All-cause and diarrhoea-associated deaths were declining since 2006, but were substantially lower since RV1 introduction and the Red Cross WASH interventions (Fig. 2 panel b). Adjusting for year to account for the longer-term trend, Poisson regression of raw monthly diarrhoea-associated mortality before and after these interventions gives mortality-rate reduction of 46% (95% CI: 26, 60) $P<0.001$.

Discussion

In this large population-based birth cohort study, national introduction of RV1 was associated with a 31% reduction in diarrhoea-associated mortality in infants surviving to at least ten weeks of age, and the degree of impact was strongly associated with vaccine coverage. Point estimate for individual protection from diarrhoea-associated mortality was 34%, though too few cases of diarrhoeal death occurred following introduction to achieve sufficiently precise confidence bounds. In the context of published RV1 impact (43%) and effectiveness (64%) estimates against laboratory-proven rotavirus hospitalization from Blantyre in Southern Malawi, our estimates of impact (31%) and effectiveness (34%) against aetiologically non-specific diarrhoea-associated death have *prima facie* validity.[6] The higher effectiveness observed in months known to have high rotavirus prevalence (January to June) and the association between vaccine coverage and impact further attest to causal plausibility. These data from a low-income, high-burden setting therefore provide compelling evidence of RV1 impact on diarrhoea-associated infant mortality.

The estimates of mortality impact in Site 1 are similar to those found in previous analyses of administrative datasets in middle-income countries.[8-10, 25] RV1 introduction in Mexico and Brazil, for example, was associated with diarrhoeal-mortality rate reduction in infants of 41% and 21% respectively.[8, 9 25] Botswana, a sub-Saharan middle-income country reported a 48% (95% CI: 11, 69) reduction in hospitalised case fatality during the rotavirus season and similar findings have been reported from Panama; though neither study measured population mortality.[26, 27] The comparable levels of protection found in our low-income Sub-Saharan African setting is encouraging, as children from this region account for more than half of global diarrhoea deaths, and with 31 African countries thus far introducing rotavirus vaccine the absolute impact on mortality is likely to be substantial. [2, 3]

The cohort design allowed us to estimate hazard and VE by age, a metric that has been approximated in case-control studies.[28] The observed hazard by age mimics the age at laboratory-confirmed rotavirus hospitalization seen in our sentinel surveillance site in Blantyre (Fig. 2 panel b). The apparent decline in VE with age is unlikely to be due to individual immunological waning before 12 months, but could be explained by changes in the force of infection through indirect effects.[12] If rotavirus prevalence is declining (Table 2), the hazard for unvaccinated infants declines, so the measurable protection afforded by vaccine direct effects is thereby reduced. Survivorship bias may also contribute to lower VE estimates in older infants, since survivors who happen to receive vaccination late do not contribute their pre-vaccination survival time to the unvaccinated cohort and survivors are implicitly more robust.

The greater individual level VE against all-cause mortality than against diarrhoea-associated mortality (Web Extra Table 2·5) in Site 1 is explained by confounding. Infants who did not receive RV1 had a greater likelihood of not receiving other EPI vaccinations, in particular pneumococcal vaccine that was introduced 10 months before RV1. Moreover such children had greater association with other socio-demographic risk factors for mortality (Appendix Table 2·5). Children from households with fewer assets had increased mortality hazard (Table 1 and Web Extra Tables 2.1-2.5). We have previously published data from Site 2 showing that vulnerable infants are at greater risk of both vaccine non-receipt and of death. [29]

Our study has several limitations. First, vaccination population-impact evaluations are subject to temporal and secular biases, particularly for aetiologically non-specific endpoints. On the other hand, individual VE estimates may be biased by access to vaccination or choice to

vaccinate. We thus sought to determine both impact and effectiveness, and took account of socio-demographic confounding. However, successful vaccines with strong impact on disease incidence challenge sufficient accumulation of cases for individual-level analysis of adequate power, because deaths become rarer events. Thus although the impact and effectiveness point estimates were similar, impact was such that effectiveness had wide confidence bounds. Second, although we inflated our sample size to account for anticipated loss to follow-up, it is possible that migrating children differed systematically from the rest of the population, thereby biasing vaccine effectiveness estimates. Single, wealthier more educated women were more mobile, but the differences, though nominally statistically significant, were modest. The observed vaccine coverage and mortality rates in the non-migrating cohort aligned with our initial expectations. Third, retrospective updating of vaccine status may have been associated with bias toward higher apparent vaccine effectiveness.[30] Coding vaccination date as date of study ascertainment rather than the date vaccination actually occurred might mitigate this bias, but this approach requires high frequency of visits. Not only is this logistically challenging in a study of this magnitude but may itself affect mortality outcome by increasing opportunity for illness recognition. Our maternal exit interviews following vaccine clinic visits showed bidirectional misclassification of about 4% (data not shown). Fourth, we went to great lengths to minimize under-ascertainment of both unvaccinated survivors and vaccinated infants who died, as previously described.[19] Yet among deceased infants health passports were often buried along with the child and unavailable for review. We could not change this cultural practice despite educational campaigns by radio and through community engagement. We actively sought vaccination clinic records to obtain vaccine status of deceased children, but it was challenging to find the correct individual records of specific infants. We therefore evaluated the quality of parental reporting through quality assurance activities. Restricting analysis to deceased infants whose records were available would itself have introduced bias. Fifth, cause

of death misclassification can affect VE. Under-reporting of diarrhoea among vaccinated deceased infants will bias VE and impact estimates away from the null. However, validation studies from Africa have shown high sensitivity for diarrhoea in VA, and these are relatively robust to recall bias, parents recollect the details of their child's final illness.[31] Sixth, since date of vaccination was not always available we could not analyse vaccination status as a time-varying covariate. This likely introduced a slight bias away from the null, since had we done so then the brief survival time between becoming eligible (we allowed 2 weeks for vaccination to be considered timely) and actually receiving vaccination would not have been included in vaccinated survival time. The fact that most vaccination was timely is therefore reassuring. Finally, other co-administered vaccines might also reduce diarrhoea-associated mortality thus subtly increasing apparent RV1 VE. Co-administration of other vaccines was almost universal, and we cannot account for this bias. In Site 2, where we report a combined impact of RV1 introduction and a comprehensive WASH intervention, the magnitude of mortality reduction was 46%. Surveillance duration and therefore model adjustments differed across our two sites so the two results are not directly comparable. Given the unanticipated co-introduction of extensive improvements in sanitation at Site 2 our result could have been biased away from the null due to other improvements in healthcare in this region, though in scoping with stakeholders we have not become aware of any other concurrent population interventions. Notwithstanding these caveats, the implication that concurrent interventions may have synergistic benefit is intriguing and warrants further programmatic evaluation.

Conclusions

Childhood diarrhoea-associated mortality in this rural African population has fallen during the past decade, in part due to improvements in sanitation and treatment interventions including ORS and zinc. Our large and comprehensive study demonstrates for the first time using

empirically observed, population-based surveillance that rotavirus vaccine further reduces diarrhoea deaths in a low income, rural African population. These data add considerable weight to the WHO recommendation that countries with high childhood mortality should add rotavirus vaccine to existing public health interventions to further reduce diarrhoea deaths.

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Author Contributions

Study conceived and designed by NF, NAC, RSH, JET, UDP, AC, CM, SL and NBZ. Data collection tools developed by NBZ, SL, ACC, JB, TP and CK. Site 1 data collection was overseen by NBZ, HM and ACC. Data management and cleaning was conducted by EH and HM. Analysis was conducted by EH and NBZ, with input from CK. Site 2 data collection was overseen by CK, JB, NBZ and TP. Data management and cleaning was conducted by CK and JB. Data analysis was conducted by CK and NBZ. Paper written by NBZ and CK with substantial input from NAC. All authors have read and commented on the final manuscript.

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Figures and Tables

Figure 1: Figure 1: Flow diagram per STROBE guidelines of participating vaccine-eligible cohort born from 17th September 2012 – 1st June 2015, Site 1

Figure 2:

Panel A. 12-month weighted moving average smoothed trend* in all-cause and diarrhoea-associated mortality in 10-51 week infants and 2-dose RV1 coverage, September 2012 to June 2015, Site 1, Malawi

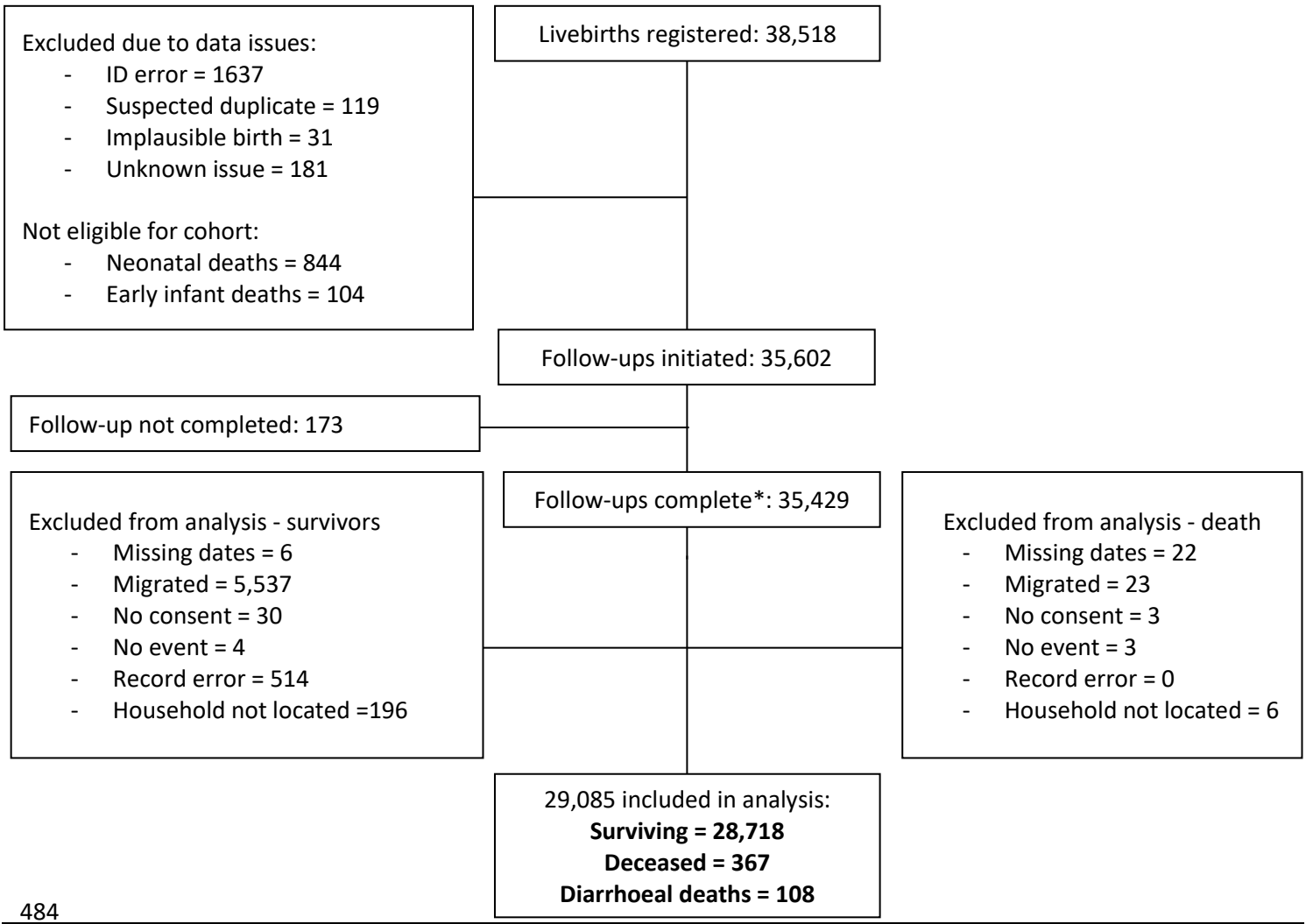
Panel B. 12-month weighted moving average smoothed trend* in all-cause and diarrhoea-associated mortality in 10-51 week infants; 2-dose RV1 coverage and 3-dose pneumococcal conjugate vaccine coverage, 2004 to 2016, Site 2, Malawi.

Figure 3: Survival analysis of diarrhoea-associated death in vaccine-eligible cohort, Site 1.

Table 1: Cohort description and multivariable Cox proportional hazards survival analysis, Site 1.

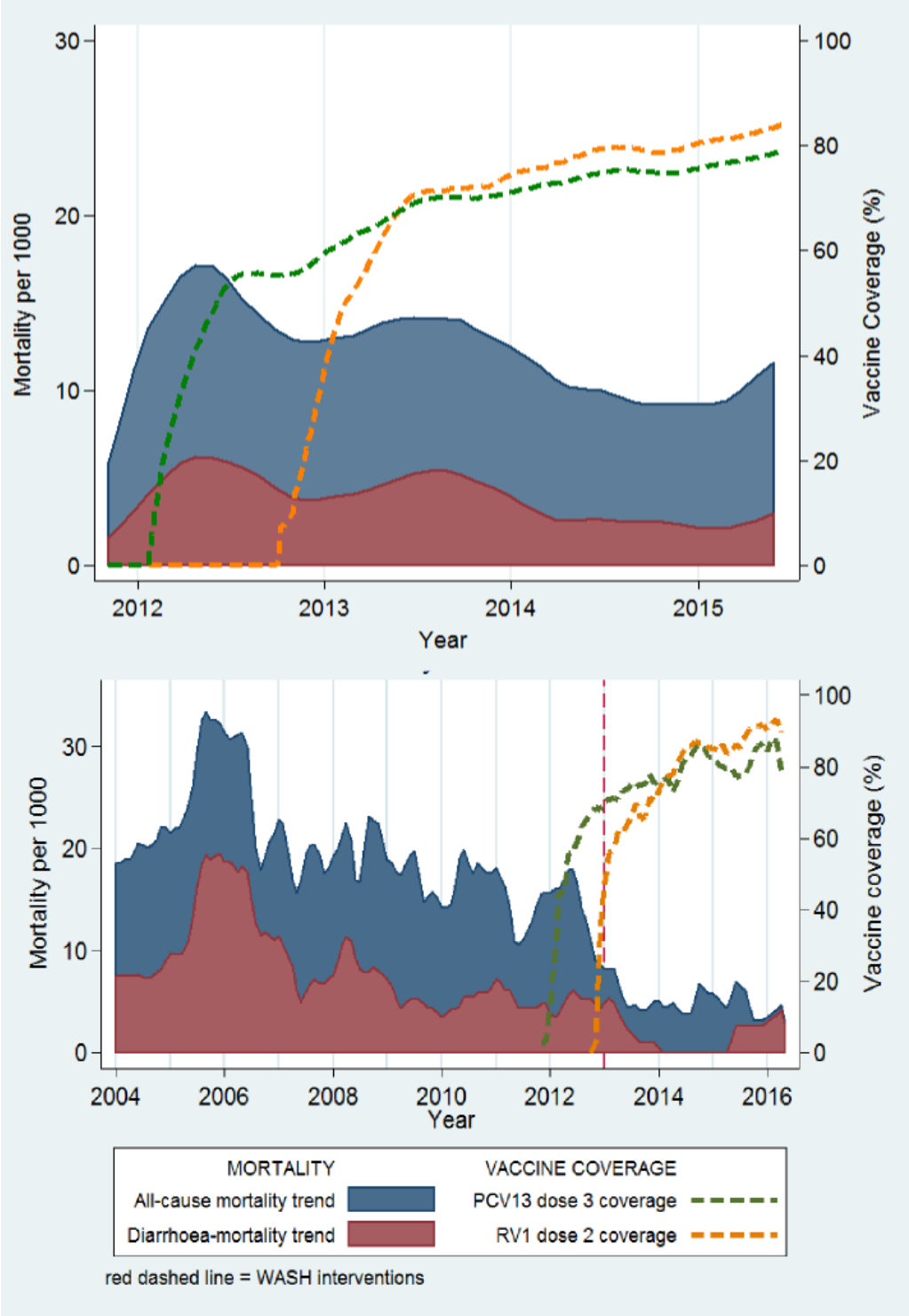
Table 2: Diarrhoea-associated death before and after RV1 introduction, Site 1.

Figure 1: Flow diagram per STROBE guidelines of participating vaccine-eligible cohort born from 17th September 2012 – 1st June 2015, Site 1



*Completion of follow-up means sufficient information was obtained by 1 year of age to determine whether the participant can be included in analysis or excluded for the reasons outlined in the figure.

Figure 2: Panel A. 12-month weighted moving average smoothed trend* in all-cause and diarrhoea-associated mortality in 10-51 week infants and 2-dose RV1 coverage, September 2012 to June 2015, Site 1. Panel B. 12-month weighted moving average smoothed trend* in all-cause and diarrhoea-associated mortality in 10-51 week infants; 2-dose RV1 coverage and 3-dose pneumococcal conjugate vaccine coverage, 2004 to 2016, Site 2.

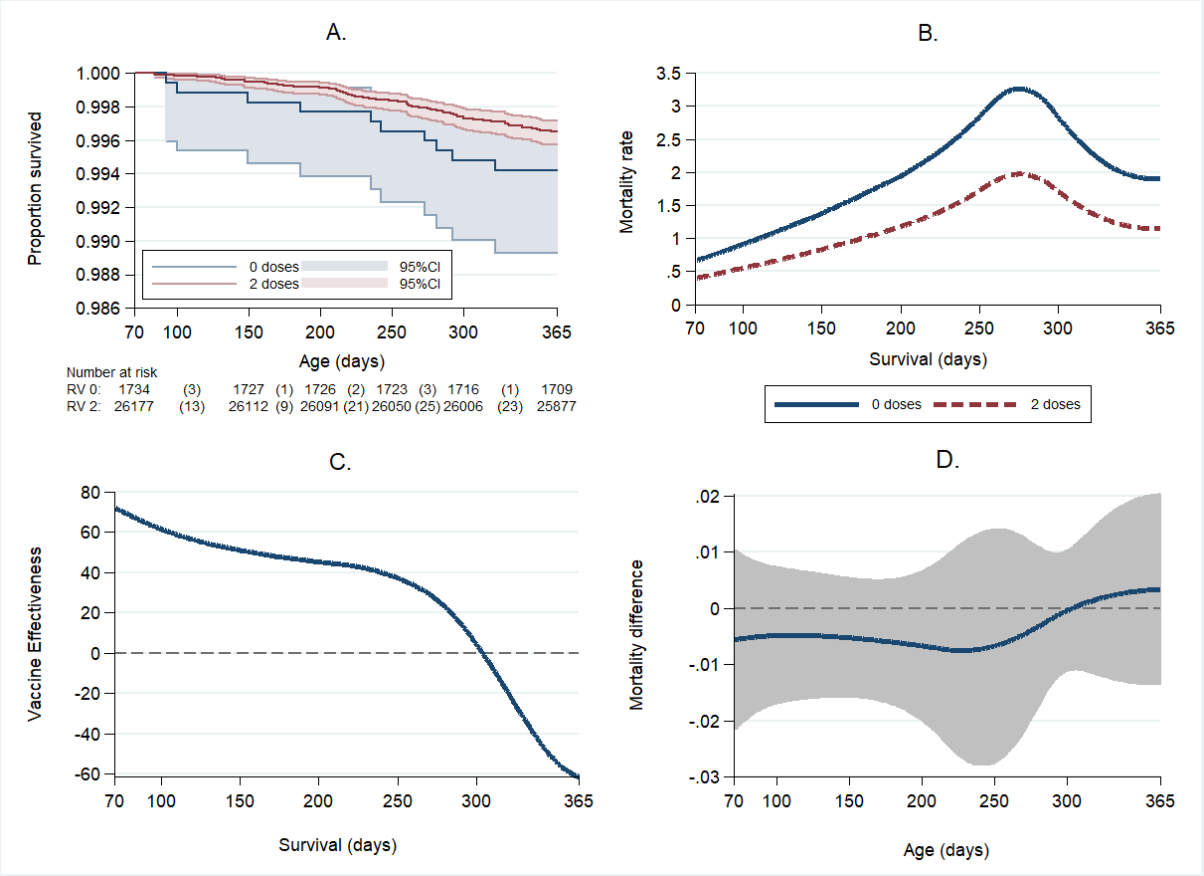


* 12-month weighted moving average smoothed trend:

$$\hat{Y}_t = \frac{1}{24}(Y_{t-6} + Y_{t+6}) + \frac{1}{12}(Y_t + Y_{t-1} + Y_{t+1} + Y_{t-2} + Y_{t+2} + Y_{t-3} + Y_{t+3} + Y_{t-4} + Y_{t+4} + Y_{t-5} + Y_{t+5})$$

; where Y_t is the monthly observation at month *t* and $\hat{Y}_t$ is the locally-weighted estimate at month *t*.

Figure 3: Survival analysis of diarrhoea-associated death in vaccine-eligible cohort, Site 1.



Panel A: Kaplan-Meier survival curve and confidence bounds, by vaccine receipt. (Deaths shown in parentheses in At-Risk table beneath the plot)
Panel B: Fully parametric hazard rate over survival time, by vaccine receipt.
Panel C: Vaccine effectiveness over survival time.
Panel D: Hazard rate difference (between vaccinated and unvaccinated infants) over survival time.

Table 1: Vaccine-eligible cohort description and multivariable Cox proportional hazards survival analysis, Site 1.

Variable		Survived		All-cause deaths		Diarrhoea-deaths		Cox multivariable model		
		N	(%)	N	(%)	N	(%)	Hazard ratio‡‡	95% CI	P-value
TOTAL		**28,718**		**367**		**108**				
Rotavirus vaccine status	0 doses	1724	(6%)	65	(18%)	10	(9%)	1		
	1 dose	563	(2%)	33	(9%)	7	(7%)	-		
	2 doses	26086	(91%)	266	(72%)	91	(84%)	0·66	0·34, 1·28	0·22
	Missing	345	(1%)	3	(1%)	-				
Maternal marital status:	Married	25810	(90%)	283	(77%)	83	(77%)	1		
	Single	1567	(5%)	39	(11%)	11	(10%)	1·91	1·00, 3·65	0·05
	Divorced/widow	1287	(5%)	33	(9%)	9	(8%)	1·55	0·74, 3·27	0·25
	Died	20	(0.1%)	9	(2%)	5	(5%)	98·1	39.5, 243.6	<0·001
	Missing	34	(0.1%)	3	(1%)	-				
Maternal education:	None	3173	(11%)	46	(13%)	13	(12%)	1		
	Primary	21963	(77%)	280	(76%)	82	(76%)	1·12	0·59, 2·11	0·73
	Secondary/Tertiary	3543	(12%)	37	(10%)	13	(12%)	0·95	0·40, 2·27	0·91
	Missing	39	(0.1%)	4	(1%)	-				
Water source	Protected source	23525	(82%)	283	(77%)	81	(75%)	1		
	Open source	5167	(18%)	81	(22%)	27	(25%)	1·42	0·90, 2·24	0·13
	Missing	26	(0.1%)	3	(1%)	-				
Toilet facility	No facility	5186	(18%)	63	(17%)	20	(19%)	1		
	Some facility	23503	(82%)	301	(82%)	88	(81%)	1·30	0·76, 2·21	0·34
	Missing	29	(0.1%)	3	(1%)	-				
House quality†	Worst	21922	(76%)	297	(81%)	86	(80%)	1		
	Middle	4302	(15%)	41	(11%)	11	(10%)	0·90	0·48, 1·72	0·76
	Best	2464	(9%)	26	(7%)	11	(10%)	1·71	0·84, 3·46	0·14
	Missing	33	(0.1%)	3	(1%)	-				
Season of birth	Dry	15229	(53%)	202	(55%)	63	(58%)	1		
	Rainy	13489	(47%)	165	(45%)	45	(42%)	0.89	0.60, 1.31	0.55
		Mean (SD)		**Mean (SD)**		**Mean (SD)**				
Mother's age††		26·0	(6·6)	27·1	(7·3)	27·9	(7·9)			
Household assets‡		1·5	(1·2)	1·2	(1·2)	1·1	(1·2)	0·72	0·59, 0·87	0·001

† House quality is a composite of the construction materials use to make the roof, walls and floor

†† Mother's age is standardized to be the age at birth of the child

‡ Household assets include: bicycle, radio, ox cart and mobile phone

‡‡ Hazard ratio of diarrhoea-associated death

509 **Table 2: Diarrhoea-associated death before and after RV1 introduction, Site 1.**

Time period	Survived	Diarrhoea-associated deaths	Diarrhoea-associated mortality rate (per 1000)	Vaccine coverage (% of eligible)	Vaccination impact * (95% CI, P-value)
Pre-vaccine cohort	7,690	44	5·6	N/A	-
Vaccine eligible cohort	28,718	108	3·7	91%	31% (1, 52, P=0.043)
Jan-Jun 2012 (pre-RV1)	4,232	28	6·6	N/A	-
Jan-Jun 2013	4,339	16	3·7	89%	39% (10, 59, P=0.013)
Jan-Jun 2014	4,180	9	2·1	94%	76% (58, 86, P<0.001)
Jan-Jun 2015	3,830	10	2·6	95%	68% (47, 81, P<0.001)

CI = confidence interval

* 1 minus relative rate reduction in mortality following vaccine introduction compared to pre-introduction rate, using adjusted Poisson regression

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Web Extra Materials

Web Extra 1: Additional figures

Web Extra Figure 1: Map of Malawi, study sites marked in red

Web Extra Figure 2: Site 1 pre-vaccination cohort flow diagram per STROBE guidelines

Web Extra Figure 3: Poisson model predicted diarrhoea-associated mortality vs vaccine coverage, Site 1.

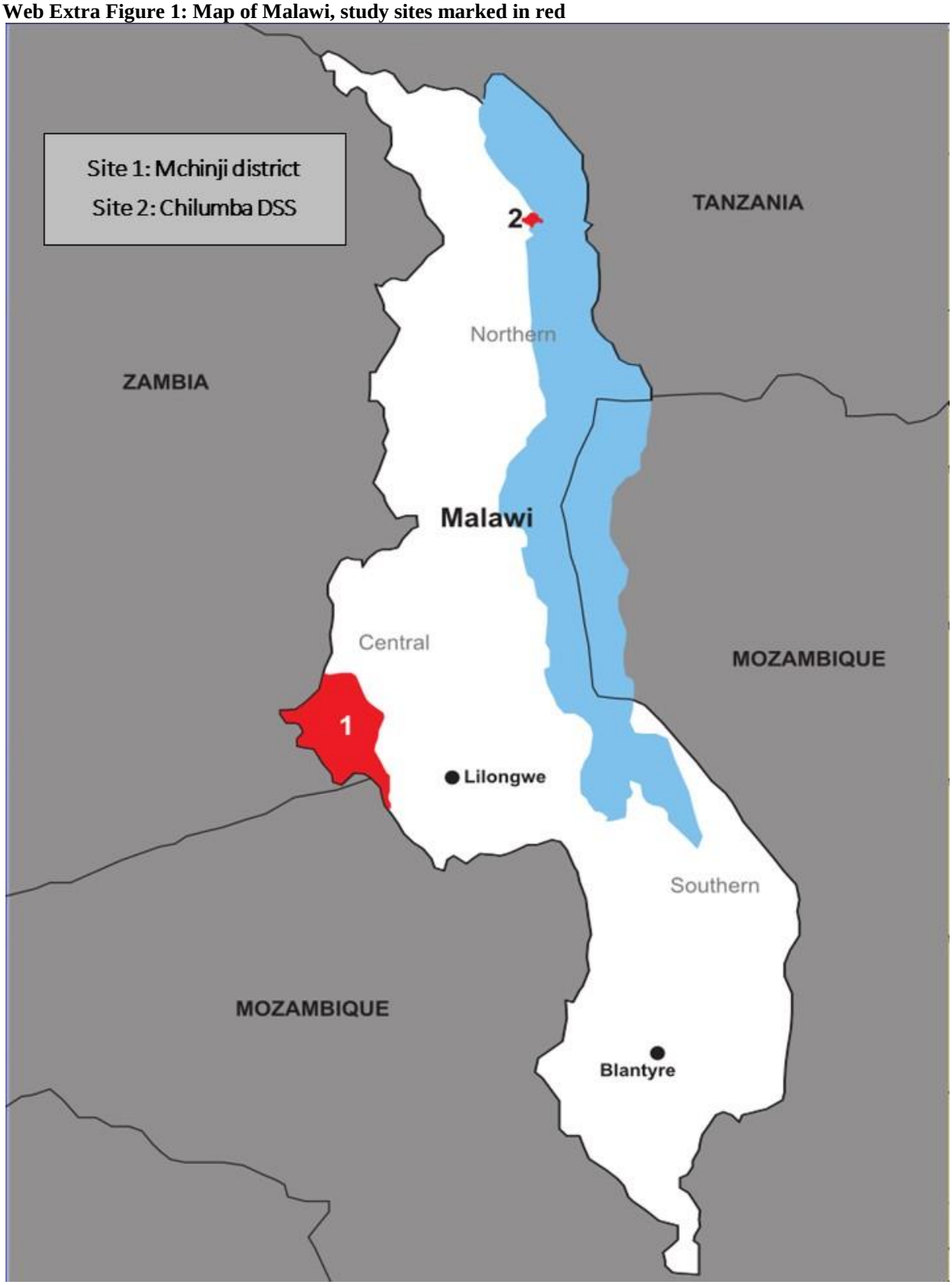
Web Extra 2: Sensitivity analysis using different survival cut-offs and investigating random effects

Web Extra 3: Socio-demographic status, Site 1.

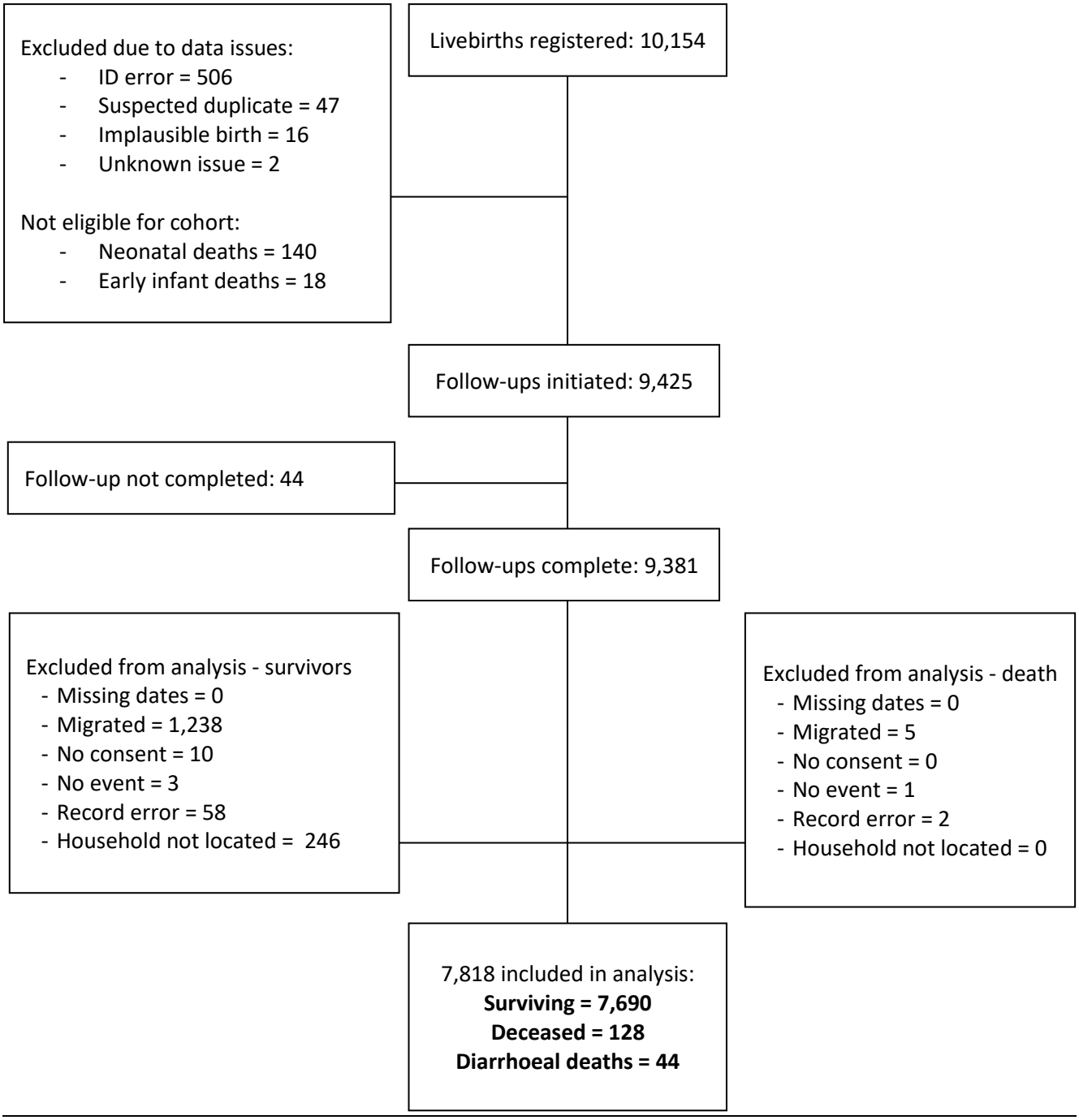
Web Extra 4: Vaccine status construction

Web Extra 1: Additional figures

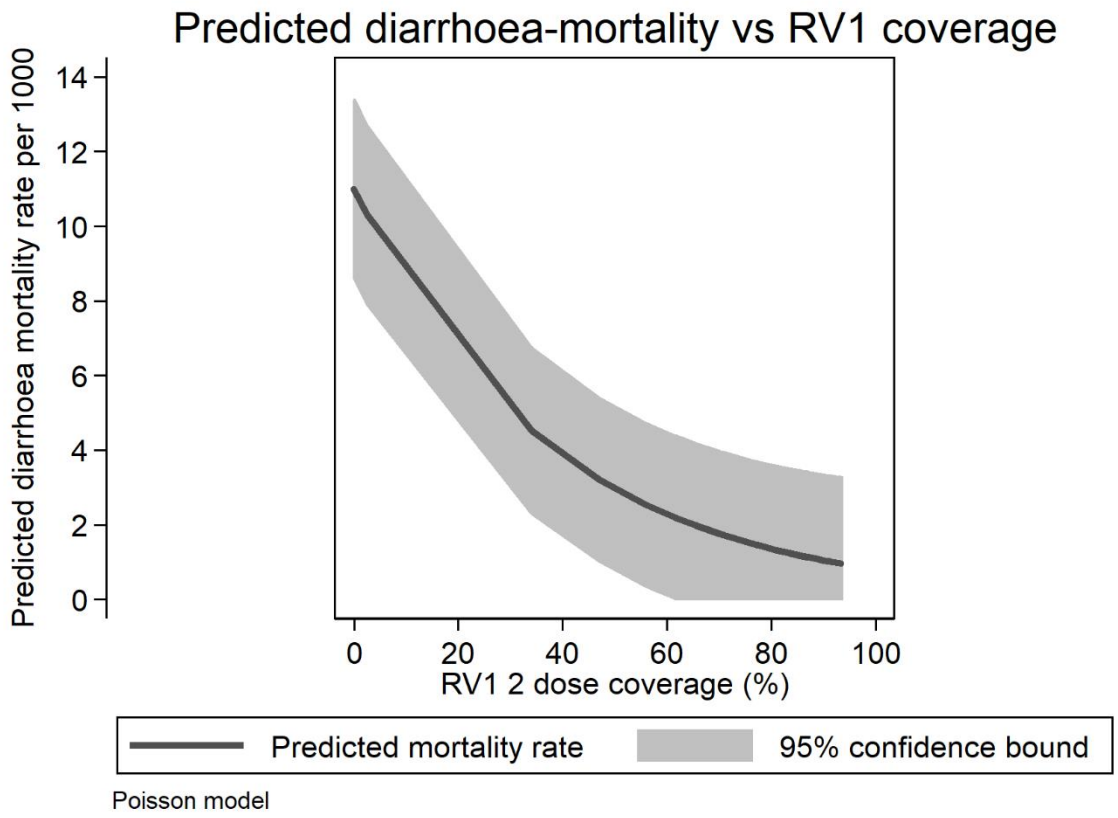
Web Extra Figure 1: Map of Malawi, study sites marked in red



Web extra Figure 2: Site 1 pre-vaccination cohort flow diagram per STROBE guidelines



Web Extra Figure 3: Poisson model predicted diarrhoea-associated mortality vs vaccine coverage, Site 1.



Web Extra 2: Sensitivity survival analyses, Site 1

2.1 InterVA defined diarrhoea outcome (10wk survival)

Variable		Hazard Ratio	95% Confidence Interval		p-value
RV status	0 doses	1·00			
	2 doses	0·71	0·21	2·35	0·574
Mother's status	Married	1·00			
	Single	2·34	0·79	6·93	0·126
	Divorced/widow	1·85	0·54	6·34	0·330
	Deceased	66·60	8·93	469·80	<0·001
Mother's education	None	1·00			
	Primary	0·57	0·23	1·42	0·230
	Secondary/Tertiary	0·44	0·10	1·89	0·271
Water source	Protected source	1·00			
	Open source	1·03	0·42	2·53	0·942
Toilet facility	None	1·00			
	Some facility	0·94	0·40	2·24	0·890
House quality	Worst	1·00			
	Middle	0·88	0·26	3·00	0·842
	Best	2·62	0·83	8·29	0·101
Household asset index		0·69	0·48	0·99	0·041

Global test of proportional hazards: 0·2942

Infants eligible for inclusion in this sensitivity analysis: 27,912 survived, 31 died

2.2 Cohort inclusion at 6 week survival

Variable		Hazard Ratio	95% Confidence Interval		p-value
RV status	0 doses	1·00			
	2 doses	0·57	0·31	1·04	0·066
Mother's status	Married	1·00			
	Single	1·85	0·97	3·52	0·061
	Divorced/widow	1·45	0·69	3·06	0·323
	Deceased	82·90	33·40	205·77	<0·001
Mother's education	None	1·00			
	Primary	1·00	0·55	1·80	0·990
	Secondary/Tertiary	0·85	0·37	1·96	0·700
Water source	Protected source	1·00			
	Open source	1·42	0·91	2·22	0·122
Toilet facility	None	1·00			
	Some facility	1·29	0·77	2·17	0·333
House quality	Worst	1·00			
	Middle	0·93	0·50	1·72	0·818
	Best	1·65	0·82	3·33	0·161
Household asset index		0·73	0·61	0·88	0·001

Global test of proportional hazards: 0·447

Infants eligible for inclusion in this sensitivity analysis: Survived = 28,342, died = 105

2.3 Cohort inclusion at 26 week survival

Variable		Hazard Ratio	95% Confidence Interval		p-value
RV status	0 doses	1·00			
	2 doses	0·72	0·33	1·58	0·412
Mother's status	Married	1·00			
	Single	1·69	0·76	3·76	0·199
	Divorced/widow	1·92	0·86	4·31	0·111
	Deceased	136·81	54·62	342·69	<0·001
Mother's education	None	1·00			
	Primary	1·17	0·56	2·47	0·675
	Secondary/Tertiary	1·08	0·40	2·89	0·881
Water source	Protected source	1·00			
	Open source	1·42	0·84	2·39	0·188
Toilet facility	None	1·00			
	Some facility	1·25	0·68	2·30	0·471
House quality	Worst	1·00			
	Middle	0·93	0·46	1·91	0·853
	Best	1·44	0·63	3·34	0·389
Household asset index		0·77	0·62	0·96	0·020

Global test of proportional hazards: 0·665

Infants eligible for inclusion in this sensitivity analysis: Survived = 27,718, died = 77

545 **2.4 Any dose of RV versus 0 doses (10 week cohort inclusion)**

Variable		Hazard Ratio	95% Confidence Interval		p-value
RV status	0 doses	1·00			
	≥1 dose	0·62	0·32	1·20	0·156
Mother's status	Married	1·00			
	Single	1·87	0·95	3·68	0·071
	Divorced/widow	1·42	0·65	3·14	0·382
	Deceased	94·73	37·47	239·49	<0·001
Mother's education	None	1·00			
	Primary	1·43	0·70	2·89	0·324
	Secondary/Tertiary	1·48	0·60	3·66	0. ·393
Water source	Protected source	1·00			
	Open source	1·56	0·99	2·44	0·053
Toilet facility	None	1·00			
	Some facility	1·24	0·73	2·12	0·422
House quality	Worst	1·00			
	Middle	0·90	0·47	1·71	0·740
	Best	1·65	0·83	3·28	0·151
Household asset index		0·77	0·63	0·93	0·006

Global test of proportional hazards: 0·779

Infants eligible for inclusion in this sensitivity analysis: Survived = 28,012, died = 101

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2.5 All-cause non-traumatic mortality (10 week cohort inclusion)

Variable		Hazard Ratio	95% Confidence Interval		p-value
RV status	0 doses	1·00			
	2 doses	0·29	0·22	0·38	<0·001
Mother's status	Married	1·00			
	Single	2·27	1·59	3·23	<0·001
	Divorced/widow	1·97	1·33	2·92	0·001
	Deceased	49·13	24·25	99·56	<0·001
Mother's education	None	1·00			
	Primary	1·02	0·73	1·45	0·89
	Secondary/Tertiary	0·75	0·45	1·24	0·26
Water source	Protected source	1·00			
	Open source	1·27	0·98	1·65	0·07
Toilet facility	None	1·00			
	Some facility	1·27	0·95	1·71	0·11
House quality	Worst	1·00			
	Middle	0·79	0·54	1·14	0·21
	Best	1·15	0·74	1·79	0·53
Household asset index		0·83	0·74	0·92	0·001

Global test of proportional hazards: 0·0002 (ie PH assumption is rejected)

Infants eligible for inclusion in this sensitivity analysis: Survived = 27,912, died = 317

Web Extra 3: Socio-demographic status, Site 1.

3.1 Socio-demographic characteristics of children according to vaccination status

Variable		0 doses N	(%)	1 dose N	(%)	2 doses N	(%)
TOTAL		1,750		603		25,831	
Mother died		3	(0.2%)	0	(0%)	22	(0.1%)
Marital status:	Married	1,536	(88%)	541	(90%)	23,273	(90%)
	Single	95	(5%)	33	(5%)	1,406	(5%)
	Divorced/widow	114	(7%)	28	(5%)	1,133	(5%)
Education:	None	260	(15%)	103	(17%)	2,762	(11%)
	Primary	1,341	(77%)	447	(74%)	19,771	(77%)
	Secondary	148	(8%)	52	(9%)	3,285	(13%)
Water source	Open source	444	(25%)	129	(21%)	4,523	(18%)
	Protected source	1,304	(75%)	474	(79%)	21,308	(82%)
Toilet facility	No facility	394	(23%)	139	(23%)	4,561	(18%)
	Some facility	1,354	(77%)	464	(77%)	21,268	(82%)
House quality	Worst	1,399	(80%)	471	(78%)	19,675	(76%)
	Middle	234	(13%)	80	(13%)	3,906	(15%)
	Best	115	(7%)	52	(9%)	2,247	(9%)
		Mean (SD)		**Mean (SD)**		**Mean (SD)**	
Mother's age		27·6	(6·76)	27·7	(7·30)	27·0	(6·58)
Household assets		1·33	(1·15)	1·36	(1·15)	1·55	(1·18)

555 **3.2 Socio-demographic characteristics of entire cohort over time**

	Year			
Socio-demographic factor	**2012**	**2013**	**2014**	**2015**
Any toilet facility	78·4%	79·9%	83·4%	85·1%
Household mobile phone ownership	38·5%	42·0%	44·6%	50·1%
No maternal education	13·6%	11·8%	10·4%	9·4%
Maternal primary education	74·2%	75·7%	77·3%	78·5%
Maternal secondary / tertiary education	11·8%	12·3%	12·3%	12·1%

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Web Extra 4: Vaccine status construction

There are three sources of vaccine status information available in Malawi:

- Health passports (government issued caregiver-held documents)
- Caregiver recall
- Under 1 government vaccine registers (filled by healthcare workers at the point of vaccination and stored in frontline health facilities)

Health passports were witnessed at home-visit interviews at 4 months and 1 year of age and at verbal autopsy interviews. Degree of reliability was then assigned to vaccine data source as outlined in the table, including relative merits of each source.

4.1 Vaccine data source reliability

Data Source	Strengths	Weaknesses	Reliability
Health passport	• Filled in at the point of vaccination • Dates included • Less than 5% mis-recording	• Differential availability according to survival status	High
Under 1 register	• Routine data, therefore should be available for all, irrespective of survival status	• Some registers are missing or of very poor quality • Issues in tracing children through registers and across facilities • Absence of record does not mean they are unvaccinated	Medium
Caregiver recall with known dates	• Dates included • Generally some documented evidence provided e.g. twins health passport	• Uncommon	High
Caregiver recall of no vaccinations	• Generally anecdotal support which makes it believable	• Uncommon • Relies on accurate recall	High
Caregiver recall	• Available for most children, regardless of survival status	• Recall bias and social-desirability bias (in both directions), so hard to adjust for the uncertainty • Chance of interviewer bias	Low

The following hierarchical rules were applied to construct a binary variable indicating vaccine received or vaccine not received:

1. If at home visit interview or VA a vaccine is recorded as 'received' in the health passport, this information will be taken as correct.
2. If at home visit interview or VA a vaccine is recorded as 'not received' or 'missing', or where no health passport was seen:
    a. If available, the vaccine status from a health passport at any prior 4-month interview (if such occurred) will be used
    b. If vaccines have been recorded in the under 1 register with evidence of a date of vaccination, this vaccine status will be used
    c. If vaccine status is not determined by 1, 2a or 2b then caregiver report will be used.
3. In case of data conflict between 4-month visit, 1 year old visit, under-1 register or maternal report, information from the health passport will be prioritised, followed by under 1 register and then caregiver report.